Colony PCR Protocol

For 1 reaction:

In a PCR tube:

25 µL Q5 2X Master Mix

1.25 µL 5' Primer

1.25 uL 3' Primer

22.5 µL H_20

Bacteria (Pick ½ of a colony off of the desired plate—there needs to be bacteria left on the plate to grow into a liquid culture later if need be.)

For multiple reactions:

In an appropriately large container:

25(X) µL Q5 2X Master Mix

1.25(X) µL 5' Primer

1.25(X) uL 3' Primer

22.5(X) µL H_20

Where X equals the number of colonies you are picking + 10% for pipetting error.

(DO NOT ADD ANY BACTERIA IF YOU WANT TO MAKE A LARGE MASTER MIX FOR MULTIPLE REACTIONS!)

Be sure to include enough of the positive and negative controls, which need to be included on each gel.

PCR Protocol:

1 cycle: 94 degrees Celsius, 5 miutes

34 cycles: 1 minute at 94 degrees Celsius, (Z)* minutes at 68 degrees Celsius, 1 minute at 72 degrees Celsius.

1 cycle: 1 minute at 72 degrees Celsius.

*(Z) = ~1 minute per kb.

Running the Gel:

Prepare a 1% agarose gel with the appropriate number of wells (multiple gels may need to be prepared for one colony PCR).

Include a ladder, a positive control, and a negative control on each gel. If a gel has been prepared with two rows of wells, make sure these rows both have a ladder, a positive control, and a negative control.

Run as much of the PCR product as desired into each well, let it run, and analyze the results.